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Description

[0001] This invention relates to the production of antibodies that are specific.

Prior Art

[0002] The fact that administering antibodies to a living animal can tolerise the animal to the antigen corresponding to the administered antibodies has been known for very many years indeed for instance see EP 0 251 065 and WO 95/17200. Treatment of pregnant women who have rhesus positive babies with antibodies to rhesus D is long established.

The invention in broad terms

[0003] The present invention resides in part in the appreciation that the theory that it is possible to switch off an immune response from naive B cells by administering antibodies to give a negative signal at their F_c receptors is not just an immunological curiosity, but can be used for substantial beneficial technical effect and has wide industrial application when it is desired to switch off an immunological response to a single antigen (or a narrow group of antigens) and yet maintain a good immunological response to other antigens. Part of the invention also resides in realising that such circumstances exist and that there is a need for such a thing.

[0004] Some aspects of the present invention allow us to realise the concept of producing antibodies specific to a particular antigenic substance (which could be part of a molecule/one or more epitopes of a molecule, or could be one antigen (or a few antigens) from a cocktail of antigens).

[0005] The invention relies upon the realisation of how to exploit technologically the antibody/antigen/naive B cell relationship, and the role of naive B cells in antibody production.

Producing Specific Antibodies

[0006] One aspect of this invention relates to the production of specific antibodies. One use for specific antibodies is test kits incorporating them.

[0007] Antibodies for test kits to look for an antigen can be produced by injecting an animal with the antigen in question, allowing its immunological response to produce antibodies to it, and then extracting the antibodies and incorporating them in a kit. This is known prior art.

[0008] If it is desired to produce an antibody to a protein that is only slightly different from another protein, for which no antibody response is required, this can be difficult to achieve. It can be impossible if the difference is very small.

[0009] A first aspect of the invention comprises a method of making antibodies to a selected part of a molecule according to claim 1.

[0010] Tolerising is achieved by introducing a compound containing the non-selected part of the molecule and antibodies to the non-selected part to the antibody source.

5 **[0011]** This is found to produce more antibodies that are specific to the selected part and do not have a response to the non-selected part.

[0012] The selected part of the molecule may be the difference between two similar molecules (e.g. allotypes), or it may be a portion only of a single molecule. Thus the non-selected part of a molecule may in fact comprise a different similar molecule (e.g. a first protein may have one or more epitopes that are different from a second molecule, and the second molecule may be the "non-selected part" for tolerisation, and the different epitopes may be the selected part to which specific antibodies are to be raised).

[0013] The antibody source is a non-human animal.

20 **[0014]** Preferably the antibody source is tolerised with all, or substantially all, of said molecule that is not said selected part. This may be achieved by tolerising with an incomplete portion of a molecule, or by tolerising with a similar molecule that has the non-selected part, but not the selected part (e.g. an allotype if the selected part is the difference between two allotypes). The antibody source may be immunised with the selected part of the molecule by injecting the whole, or substantially the whole, molecule into it. Alternatively substantially only the selected part may be immunised.

30 **[0015]** It will be appreciated that tolerising the immunological antibodies to a non-selected part of a first molecule may be achieved by introducing antibodies to the non-selected fragment of the first molecule in question, or by introducing antibodies to another molecule, a second molecule, that has the non-selected part of the first molecule in question plus another part or parts (but not the selected part of the first molecule).

35 **[0016]** This effectively augments the production of antibodies of the system to the difference between the two molecules (or between the tolerised fragment of molecule and the selected part). This can help produce a good production of antibodies to one allotype, but not another.

40 **[0017]** The antibody source that is used to produce the antibody to the non-selected part is not the same source (animal) that is later used to produce the antibody to the selected part. That is to say a different source (animal of the same species) has the antibody to the non-selected part used to tolerise it for the production of antibody to the selected part.

50 **[0018]** The animal, or other antibody source, chosen to produce the antibody to the selected part should preferably not have been immunised to the non-selected part beforehand. This is because once an immunological response has been established in a particular animal it will have memory B cells which cannot be switched off by introducing antibodies to the protein in question (non-selected part) and so the animal when immunised with

the selected part (as part of the protein used to immunise) will produce antibodies to both the selected part and the non-selected part. The yield of antibodies to the selected part will be less, and there will be two different antibodies which will need to be separated. This is all best avoided by ensuring that the animal to which the selected part immunising injection is given has not already been immunised to the non-selected part (and clearly this includes the entire protein which would contain the selected and non-selected parts).

[0019] The antibody to the selected part and/or non-selected part may be extracted from the animal in its blood or from some other substance or material produced by the animal. We may extract about a pint of blood from an animal such as a sheep.

[0020] Preferably the antibody to the non-selected part is purified before being introduced to the animal that is to produce the antibody to the selected part. The purification is preferably an affinity purification. If there is a wider mix of antibodies in the extract from the animal purification is more difficult. By preferentially producing antibodies of substantially only one kind the difficulty of the purification stage is reduced.

[0021] The animal (or other antibody source) that is to produce the antibody to the desired part may be tolerised to the non-selected protein part (or antibodies to that part) at the same time as it is immunised with the selected part, or beforehand, or afterwards. Any time within a day or so either side of immunisation is seen as being a suitable time for tolerisation.

[0022] It will be appreciated that the present invention allows the production of polyclonal antibodies faster, and to only a part of a naturally occurring molecule, and enables a greater concentration in the animal extract of antibodies of the desired kind to be obtained.

[0023] The same invention can be used to stimulate the antibody response to one desired antigen from, for example, an impure antigen injection (where there are other antigens as well as the desired one).

[0024] The claimed method may be used in a method of making antibodies to a selected antigen (first antigen) selected from a plurality of antigens (other antigens) to which an antibody source is exposed, the method comprising tolerising the immunological system of the antibody source to said other antigens, and not to said first antigen; and immunising the antibody source with said first antigen, allowing the antibody source to produce antibodies to said first antigen; and extracting the antibodies produced from the antibody source.

[0025] According to the invention, the antibody source has administered to it an antigen comprising the non-selected epitope (s), or "other" antigens as the case may be, before antibodies to the non-selected epitopes (s), or "other" antigens, are administered (and the selected or first antigen). This leads to an even better production of the antibodies to the selected epitope, or to the first antigen, as the case may be. We may administer the antigen (non-selected epitopes (s)), or "other" antigens

at least 1 or 2 days and preferably 3 days, before tolerising with the unwanted antibodies.

[0026] A test kit having a preparation that contains antibodies to a selected part, or epitope, of a protein, the antibodies having been made in accordance with the first aspect of the invention is foreseen.

[0027] Preferably the test kit is an immunological test kit.

[0028] The antibodies may be linked to an adjuvant, or to a particle or microsphere, such as a polystyrene microsphere.

[0029] Hitherto kits which were specific to part only of a protein have been difficult to make, and produce results which are not satisfactory. Preferably the protein is naturally occurring, most preferably in man.

[0030] The kit may contain instructions on how to use it. It may contain a bottle of control material, and/or a bottle of reference standard material in addition to the bottle of antibody material. The containers of antibodies, control material, and standard reference material will, if provided, usually be labelled as such. It is envisaged that the antibodies in the kit would be provided in a preparation that has between 1-5 mg/l of antibodies.

[0031] The kit is preferably suitable to be used on automated test apparatus (of course the kit could be used manually, but it is preferably sensitive enough to allow the test to be run on existing automated laboratory systems).

[0032] When treating a patient (or considering beginning treatment) a diagnostic test may be performed before a decision is made.

[0033] The skilled practitioner in the field of antibody production, possibly in consultation with an expert in splitting molecules at a desired place, or binding molecules to a particular region, should be able to produce an antigen with the active part masked, or removed, without any undue difficulty. Some trial and error may be required to find the best excision point, or the best way of masking the active region, but no further invention will be required.

[0034] In the case where we tolerise to the whole of a substance the skilled man has no difficulty in producing, and purifying, antibodies (e.g. sheep or human, or other mammal) to a substance.

Suppressing Immune Response to Foreign Substances by Associating the Substance with an Antigen and Ensuring that Antibodies to that Antigen are Present

[0035] We have realised that it is possible to switch off B-cells by triggering their Fc receptors with antibodies to the antigen we want to introduce deliberately.

Possible Theory to explain the invention

[0036] We believe that it will be helpful to discuss a possible theory relating to the mechanism operating in

the immune system which may help to explain the invention. We should, however, make it clear that this is only a theory: the invention works and we want protection for the features of the invention we have disclosed. Even if in the end a different mechanism operates we still have disclosed to the world practical steps to enable the technical effects of the invention to be obtained and are entitled to patent protection for that.

[0037] We will illustrate the theory in relation to some examples discussed with reference to the accompanying drawings of which:-

Figure 1 schematically illustrates a naive B-cell being stimulated by an antigen;

Figure 2 schematically shows a different naive B-cell being inhibited from producing anti-IgG2 antibodies by the presence of antibodies to a common epitope

Figure 3 shows a naive B-cell being inhibited from producing anti-IgG1 antibodies by the presence of antibodies to a common epitope;

Figure 4 shows schematically a whole IgG and a free lambda;

Figure 5 shows B-cell inhibition preventing antibodies to free lambda being formed by the delivery of a negative signal to the F_c receptor by an anti-exposed determinant antibody binding to the free lambda, and to the F_c receptor;

Figure 6 shows B-cell inhibition to the production of exposed determinant antibodies by the presence of anti-exposed determined antibodies, ensuring that B-cells for such production are not stimulated, whereas B-cells for hidden determinants may still be stimulated;

Figure 7 shows schematically an active form of an enzyme and an inactive form of the enzyme having common epitopes;

Figure 8 shows a B-cell which would produce antibodies specific to the epitope exclusive to the active form of the enzyme if it were not inhibited by antibodies to a common epitope binding to the common epitope and the F_c receptor;

Figure 9 shows a modified form of the enzyme, having albumin coupled to it; and

Figure 10 shows the inhibition of B-cells which recognise common epitopes by administering anti-albumin.

Example 1: Producing specific antibodies to IgG1.

[0038]

1. Every molecule of IgG1 (G1) has G1 specific epitopes as well as epitopes that are common to all the others subclasses. If we immunise solely with G1 (plus adjuvant) then the antibodies produced will react to both the G1 specific epitopes plus the common epitopes.

2. If we inject intravenously with a preparation of pure G2, G3 and G4 (in the absence of adjuvant) before the time of immunisation, we get a tolerisation effect whereby the generation of antibodies to the common epitopes is partially inhibited.

3. We have subsequently found that intravenous administration of antibodies to G2, G3 and G4 alone at the time of immunisation also reduces the immune response to the common epitopes.

4. If 2. and 3. above are combined, i.e. giving pure G2, G3 and G4 three days before immunisation, and giving antibodies to G2, G3 and G4 at the time of immunisation, then the inhibition of the immune response to the common epitopes is strongly enhanced, and only antibodies to the G1 specific epitopes are produced.

[0039] Typically, as shown in Figure 1, a resting B-cell 10 with an antigen receptor 11 recognising a specific G1 epitope 12 will bind to G1, and will be stimulated to produce antibodies to the specific G1 epitope. Similarly, resting B-cells with an antigen receptor to a common epitope 13 on G1 will be stimulated to produce antibodies to the common epitope (which therefore will cross-react with G2 and/or G3 and/or G4).

[0040] We can use the administration of specific antibodies to the other subclasses to decrease or inhibit the response to common epitopes on the target antigen (in this case G1). The mechanism of this is shown in Figure 2. When G2 is administered (as a tolerogen 9) this is bound by the antigen receptor 14 of any B-cells 15 present that recognise the common epitope. This would normally stimulate the B-cell to produce antibodies to the common epitope. In this case however, subsequent administration of antibodies 16 specific to G2 leads to inhibition of antibody production of the B-cell. The mechanism by which this is thought to be mediated is through the binding of antibody to the G2 tolerogen, and the interaction of the antibody and the B-cell receptor (see Figure 2). In this way, all naive B-cells recognising common epitope are inhibited, so that if G1 is given at the same time as the specific anti-G2, only B-cells recognising specific G1 epitopes are available to be stimulated. Thus only antibodies specific for G1 (and not to common epitope) are produced.

[0041] We prefer to have the tolerogen dose much greater than the immunogen dose, therefore B-cells recognising common epitopes will be "eliminated".

[0042] The tolerogens do not themselves elicit an immune response as they are administered intravenously and in the absence of adjuvant.

[0043] A relevant aspect of this process is that pure antibodies to G2, G3 and G4 are required; if these antibodies are impure (i.e. would also react with the common epitopes), then naive B cells recognising G1 would also be turned off as in Fig 3. This has been demonstrated experimentally by immunising sheep (previously tolerised with pure G2, G3 and G4) with IgG1 and at the same time administering antibodies to the common epitopes. A poor response to G1 was obtained.

Example 2: Producing specific antibodies to free lambda.

[0044] The requirement here is to make antibodies to the "hidden" epitopes 40 (which are only exposed when lambda is in the free form) and not the "exposed" epitopes 41 that are always present (on the "free" and "bound" forms of lambda). In this case the epitopes are on the same molecule, but on different sides (see Figure 4).

[0045] Here an approach could be to raise antibodies to the exposed determinants 41, then administer these when immunising with free lambda 42. The presence of antibodies to the exposed determinants should inhibit production of antibodies to exposed determinants, allowing only antibodies to the hidden determinants to be produced. However, due to the positioning of the epitopes, some inhibition of the response to the hidden epitopes can also occur (see Figure 5).

[0046] The above dichotomy has been demonstrated experimentally; the antibodies produced following the tolerising regime are more specific than those produced using a normal immunisation procedure, but they are of lower affinity. To overcome this, it is proposed to administer whole IgG as the tolerogen, then give anti-IgG Fc at the time of immunisation with free lambda (see Figure 6). This will inhibit all B cells recognising exposed lambda epitopes, so that only B cells recognising hidden lambda epitopes will be stimulated. Thus, only antibodies recognising hidden lambda epitopes will be produced. Experimental results consistent with this have been achieved.

Example 3: Producing specific antibodies to the active form of an enzyme.

[0047] This follows the same basic principle used in Example 2. In this case it is desired to produce antibodies that react only with the active form 70 of an enzyme, and not with any of the common epitopes 71, 72, that are present on both the active (70) and inactive (73) forms.

[0048] One initial approach could be to raise antibodies to the inactive enzyme 73, and use these to tolerise. However, when immunising with the active form of the enzyme, B cells recognising the active form epitopes 74 are likely to be inhibited in an analogous situation to that shown in Figure 5 (see Figure 8).

[0049] This problem can be overcome by conjugating a suitable carrier protein (e.g. albumin) to the inactive enzyme (see Figure 9) and then administering this as a tolerogen. The active enzyme would then be given as the immunogen, together with anti-albumin. This would result in inhibition of B cells recognising common epitopes - see Figure 10. Thus, only B cells recognising active form epitopes 74 would be stimulated, resulting in active form specific antibodies being produced.

Producing Specific Antibodies Examples

[0050] Having now described the three general areas in which we see the present invention having applications, and having discussed a theory which we believe explains the present invention, we shall now describe specific examples in relation to each, by way of example only, with reference to the accompanying drawings of which:-

Figure 11a shows a paraprotein having light chains bound to its heavy chains;

Figure 11b shows the light chains of Figure 1a with its hidden determinants exposed;

[0051] Multiple myeloma can be detected by looking for paraproteins in the urine of patients. In normal people tiny amounts of paraprotein are present in the blood. Figure 11a shows schematically a paraprotein. If plasma cells (bone cells) are de-differentiated they can produce a part of the paraprotein, the light chains. These are much smaller than the whole paraprotein (25kD cf. 150kD) and are excreted into urine by the kidneys. Figure 11b schematically shows the lambda light chains of the paraprotein of Figure 11a. Thus a multiple myeloma can be detected by noticing high levels of kappa and/or lambda light chains of the plasma cells. At present the presence of the light chains is detected using antibody binding and electrophoretic techniques. The antibodies used react with free light chains, and also with light chains bound to heavy chains. This can mean that the test can show a positive result for whole paraproteins, which might be present for a variety of reasons not necessarily associated with myeloma (e.g. kidney damage can allow the whole paraproteins to pass through).

[0052] In this example the present invention comprises producing antibodies that are specific to the hidden determinants of the light chains (which will not be available for binding in the whole paraprotein, only when there are free light chains).

[0053] The production of such specific antibodies can

be enhanced by tolerising the host antibody-producing animal with antibodies to the remainder of the paraprotein - the exposed determinants (i.e. tolerise with paraprotein with bound light chains), and immunising the animal with free light chains of the paraprotein. This causes in the host animal those B-cells that recognise the exposed determinants to be switched off, leaving only B-cells recognising the hidden determinants; thus only antibodies to the hidden determinants are produced.

[0054] These hidden determinant specific antibodies are then extracted from the host animal and used in the production of kits for immunological assays. The kits can be used to detect the free light chains in urine, or even in blood.

[0055] The process enables the production of high affinity polyclonal antisera to free kappa and lambda light chains. These antisera find uses in tests and test kits.

[0056] What it is proposed to do in practice is in one example to immunise one animal (e.g. a sheep, sheep A) with a non-selected part of a protein. The antibody to the non-selected protein would then be extracted from the animal's blood (eg a pint of blood would be taken, with the sheep still in good health) and purified with an affinity purification technique.

[0057] Another sheep, sheep B, that had not been immunised with the whole protein, nor with the non-selected part of the protein, would be taken and injected with the antibodies from sheep A that react only with the unwanted, non-selected, part and also, at the same time, the antigen that is whole of the protein that the selected part forms a part of (in the case of paraprotein with the free light chains).

[0058] Antibodies from the blood of sheep B would then be extracted (eg 100 ml to 1 litre of blood taken, leaving the sheep in good health), and these would be antibodies which were specific to the selected part of the protein. They would be used in a kit. Alternatively they might first be purified and then used in a diagnostic kit.

[0059] It will be appreciated that instead of injecting the whole of the protein that the selected part forms part of it is possible to consider injecting just the selected part, but that would be more expensive to make, and may not even be possible.

[0060] It will be appreciated that one main use of antibodies made in accordance with the present invention is in an immuno assay for measuring free immunoglobulin light chains, preferably in automated test apparatus. The example discussed in relation to Figures 11a and 11b was to look for multiple myeloma, but the technique is also, of course, suitable for testing for other paraproteinaemias.

[0061] In addition to performing a test to detect whether a patient has a paraproteinaemia tests would also be performed to monitor the patient periodically.

[0062] It is a known technique to attach antibodies to particles in order to improve the sensitivity of a test. It is possible that the antibodies produced would be linked

to a particle (e.g. a polystyrene microsphere). This may be another stage in the production of test antisera.

[0063] It will be appreciated that in the example of Figures 11a and 11b a specific antibody to a single part of a molecule, and not to other parts of the molecule (or to the difference between two similar molecules) is produced.

[0064] This invention has wide application. For example, applying the same concept to an enzyme allows the production of an antibody specific to the active site of the enzyme (e.g. by tolerising with the enzyme in inactivated state, and immunising with the enzyme in activated configuration).

[0065] As discussed in relation to Figures 7 to 10 it is possible to immunise to produce antibodies specific to the difference between two states of a molecule. It is possible to produce antibodies only to the active form of an enzyme, or molecule, and not the inactive form (or substantially only to the one form). This can be used to produce a functional assay (one that looks at whether a particular enzyme is functional). In this case the "selected part" of the molecule could be the active site in its active configuration, and the non-selected part could be the entire inactive molecule.

[0066] One particular area where the invention may have a commercial use is in the production of catalytic antibodies. It is known that antibodies can act as catalysts in chemical reactions: just as enzymes catalyse reactions some antibodies can also catalyse a specific reaction. It is believed that catalytic antibodies bind one or more of the reaction molecules at a "pocket" or binding site and force it to have the right shape/charge distribution to undergo the chemical reaction that the antibody catalyses. In the production of a catalytic antibody a template antigen of an appropriate shape/size and charge is used to produce an antibody to it. The present invention allows catalytic antibodies to be produced better by, for example, tolerising with antibodies to non-template antigen (or with the non-template antigen itself) and then immunising with the template antigen. It is preferred to tolerise with antibodies to the non-template antigen (or antigens) since it is believed that this gives better antibodies to the template antigen.

[0067] Of course, a catalytic antibody produced using the present invention need not be used in a blood or urine test kit. It may be used in vivo to catalyse its reaction in order to obtain the reaction products (or destroy the reaction starting materials). Alternatively it could be used in vitro to catalyse the reaction in question. Indeed, this might be one way of helping some people or animals that have a defective enzyme in their metabolism.

[0068] The idea of using tolerisation/immunising to produce an enhanced response to a specific part, or epitope, is a tool to exploiting the activity of that part, or epitope, and/or antibody.

[0069] It has been appreciated that if the B cells to a part of a molecule are switched off it is possible to get a good antibody response to the other part of the mole-

cule. Limiting the response to specific molecules in an antigen mixture by administering antibodies to the undesired antigens at the time of immunisation is how it is preferred to produce the desired antibodies.

[0070] Returning to the example of Figures 1a and 1b this is the first time that it has been possible to produce polyclonal antisera against hidden epitopes on single molecules.

[0071] In addition to using the invention to produce antibodies to a part only of a molecule it can be used to produce antibodies to one molecule, but not another similar, but different molecule (different allotypes). This enables us to provide a sensitive probe to different allotypes.

[0072] It will be appreciated that the method of diagnosis will not normally be practised on a human or animal body, but rather on a sample (e.g. blood, urine, tissue) extracted from the body.

[0073] It will be appreciated that the present invention allows the production of antibodies faster, and to only a part of a molecule, and enables a greater concentration in the animal extract of antibodies of the desired kind to be obtained.

[0074] The selected part of the molecule (for which antibodies are not required) may be substantially all of the molecule except the non-selected (or de-selected) part. If the non-selected part of the molecule is the part that gives it its physiological effect we can make antibodies to all of the molecule except the physiologically active part. This is very convenient from the point of view of providing a range of antibodies to tolerate a patient to allow repeated administrations of the molecule.

[0075] We may ensure that we tolerate with low affinity antibodies to the selected part of the molecule.

[0076] We may tolerate with low affinity antibodies to the non-selective parts of the molecule, or with high affinity antibodies.

[0077] The level of antibodies that we give to tolerate is, as will be appreciated, very low. We might inject 1,2,3,4,5,6,7,8,9,10 or more microgrammes of antibody. We do not need to inject much, just enough to switch off the B cells (or many of them).

[0078] It will be appreciated when we want to make an antibody specific to a selected part of a molecule we can tolerate to the immunological antibodies to a non-selected part of a first molecule by introducing antibodies to the non-selected fragment of the first molecule in question, or by introducing antibodies to another molecule, a second molecule, that has the non-selected part of the first molecule in question plus another part or parts (but not the selected part of the first molecule). The antibodies to the non-selected part and/or to the selected part are preferably extracted from an animal in a way which still leaves the animal alive, possibly for further harvesting of antibodies. For example, an animal could be bled and the antibodies extracted from its blood. It is quite possible to take 100ml to 1 litre of blood from many animals without doing them permanent harm, and leave

the animal available for further antibody harvests in the future. The antibody source that is used to produce the antibody to the non-selected part is preferably not the same source (animal) that is later used to produce the antibody to the selected part. That is to say a different source (animal of the same species) has the antibody to the non-selected part used to tolerate it for the production of antibody to the selected part.

[0079] The antibodies that we introduce preferably have a high affinity for the proteins they attach to. We may prefer to have low affinity antibodies, if there are any antibodies at all, to the selected site.

[0080] We prefer to inject the selected part of the molecule and the antibodies to it at about the same site on the human or animal (possibly sequentially, or simultaneously). We may prefer to inject the selected part substance at one site and the antibodies at another. The two sites may be well spaced apart. One or both of the sites may be subcutaneous, intravascular, or intramuscular.

Claims

1. A method of making antibodies to a selected part of a molecule comprising tolerising the immunological system of a first non-human animal source of antibodies to a non-selected part of said molecule by presenting to the immunological system of the first source a compound containing the non-selected part of the molecule, and also presenting to the first source, antibodies to the non-selected part of the molecule, and immunising the first source of antibodies with the whole molecule, i.e. the selected part of the molecule together with the parts used for tolerisation, allowing the first source of antibodies to produce antibodies specific to said selected part of the molecule, and extracting the antibodies produced from the first source and in which the second source that is used to produce the antibody to the non-selected part is not the same individual first source that is later used to produce the antibody to the selected part, and the first source chosen to produce the antibody to the selected part has not been immunised to the non-selected part beforehand.
2. A method of making antibodies according to claim 1 which comprises tolerising the immunological system of the first source to the non-selected part of the molecule by immunising a second non-human animal source of antibodies with the non-selected part, or a whole, of the molecule to raise an antibody to the non-selected part, extracting the antibody to the non-selected part, and using the antibody to the non-selected part to tolerate a first source of antibodies to enhance the production of antibodies to the selected part.

3. A method according to claim 1 or claim 2 in which blood or other substance material produced by the non-human animal first source is extracted and the antibody to the selected or non selected part is extracted from said blood or other substance material. 5
4. A method according to any one of the preceding claims in which the antibodies made are polyclonal antibodies. 10
5. A method according to any one of the preceding claims in which the selected part comprises the difference between two similar molecules, and in which the non-selected part that is used to tolerise is the whole of one molecule (or substantially the whole), and the immunising step is achieved by introducing to the source the whole of the other molecule (or substantially the whole). 15
6. A method of making antibodies as claimed in claim 1 against a first immunoglobulin G subclass from the group IgG1, IgG2, IgG3 and IgG4 which method comprises tolerising the immunological system of a first non-human source of antibodies to the other immunoglobulin subclasses of the immunoglobulin and also presenting to the first source, antibodies to the other immunoglobulin subclasses of the immunoglobulin and immunising the first source of antibodies with the first immunoglobulin subclass allowing the first source of antibodies to produce antibodies specific to said first immunoglobulin subclass, and extracting the antibodies from the first source. 20 25 30
7. A method according to any one of claims 1-5 in which the selected part of the molecule comprises an activated site of an enzyme. 35
8. A method of making antibodies according to any one of the preceding claims in which the antibodies to the non-selected part are injected intravenously. 40
9. A method of making antibodies according to claim 4 or any claim dependent directly or indirectly from claim 4 in which the antibody is extracted from the blood or other substance or material is extracted in such a way which leaves the animal alive. 45
10. A method of making antibodies specific for epitopes on kappa or lambda light chains which are hidden and will not be available for binding in the whole immunoglobulin but are exposed on free light chains comprising tolerising the immunological system of a first non-human animal source of antibodies with immunoglobulin with bound light chain and with antibodies to the exposed epitopes of the immunoglobulin, and immunising the first source of antibodies with free light chain and allowing the first 50 55

source of antibodies to produce antibodies specific for the free light chain, and extracting antibodies produced from the first source, and in which the second source that is used to produce the antibody to the exposed epitopes of the immunoglobulin is not the same individual non-human animal source as the first source that is later used to produce the antibody to the free chains, and in which the first source chosen to produce the antibody to the free chain has not been immunised with the immunoglobulin before hand.

Patentansprüche

1. Verfahren zum Herstellen von Antikörpern gegen einen selektierten Teil eines Moleküls, umfassend Tolerantmachen des immunologischen Systems einer ersten nichtmenschlichen Tierquelle von Antikörpern gegen einen nicht-selektierten Teil des Moleküls durch Präsentieren einer Verbindung, welche den nicht-selektierten Teils des Moleküls enthält, dem immunologischen System der ersten Quelle, und ebenfalls Präsentieren, der ersten Quelle, von Antikörpern gegen den nicht-selektierten Teil des Moleküls, und Immunisieren der ersten Quelle von Antikörpern mit dem Gesamtmolekül, d.h. den selektierten Teil des Moleküls, gemeinsam mit den für die Tolerantmachung verwendeten Teilen, was der ersten Antikörperquelle gestattet, gegen den selektierten Teil des Moleküls spezifische Antikörper zu produzieren, und Extrahieren der von der ersten Quelle produzierten Antikörper; wobei die zweite Quelle, die verwendet wird, um den Antikörper gegen den nicht-selektierten Teil zu produzieren, nicht dieselbe individuelle erste Quelle ist, die später verwendet wird, um den Antikörper gegen den selektierten Teil zu produzieren und die zum Produzieren des Antikörpers gegen den selektierten Teil gewählte erste Quelle nicht zuvor gegen den nicht-selektierten Teil immunisiert worden ist.
2. Verfahren zum Herstellen von Antikörpern gemäß Anspruch 1, welches umfasst: Tolerantmachen des immunologischen Systems der ersten Quelle gegen den nicht-selektierten Teil des Moleküls durch Immunisieren einer zweiten nichtmenschlichen Tierquelle von Antikörpern mit dem nicht-selektierten Teil oder dem gesamten Molekül, um einen Antikörper gegen den nicht-selektierten Teil zu ziehen, Extrahieren des Antikörpers gegen den nicht-selektierten Teil und Verwenden des Antikörpers gegen den nicht-selektierten Teil, um eine erste Quelle von Antikörpern tolerant zu machen, um die Herstellung von Antikörpern gegen den selektierten Teil zu verstärken.
3. Verfahren gemäß Anspruch 1 oder Anspruch 2, bei

dem Blut oder Material einer anderen Substanz, das von der ersten nichtmenschlichen Tierquelle hergestellt wird, extrahiert wird und der Antikörper gegen den selektierten oder nicht-selektierten Teil aus dem Blut oder dem Material, anderer Substanz extrahiert wird.

4. Verfahren gemäß einem der vorstehenden Ansprüche, bei dem die hergestellten Antikörper polyklonale Antikörper sind.
5. Verfahren gemäß einem der vorstehenden Ansprüche, bei dem der selektierte Teil den Unterschied zwischen zwei ähnlichen Molekülen umfasst und bei dem der nicht-selektierte Teil, der zum Tolerantmachen verwendet wird, das gesamte Molekül (oder im wesentlichen das gesamte) ist und der Immunisierungsschritt durch Einführen des gesamten anderen Moleküls (oder im wesentlichen des gesamten) in die Quelle erreicht wird.
6. Verfahren zum Herstellen von Antikörpern gemäß Anspruch 1 gegen eine erste Immunglobulin G-Unterklasse aus der Gruppe IgG1, IgG2, IgG3 und IgG4, wobei das Verfahren umfasst: Tolerantmachen des Immunsystems einer ersten nichtmenschlichen Quelle von Antikörpern gegen die anderen Immunglobulin-Unterklassen des Immunglobulins und ebenfalls Präsentieren von Antikörpern gegen die anderen Immunglobulin-Unterklassen des Immunglobulins der ersten Quelle und Immunisieren der ersten Quelle von Antikörpern mit der ersten Immunglobulin-Unterklasse, was es der ersten Quelle von Antikörpern gestattet, Antikörper zu erzeugen, die für die erste Immunglobulin-Unterklasse spezifisch sind, und Extrahieren der Antikörper aus der ersten Quelle.
7. Verfahren gemäß einem der Ansprüche 1 bis 5, bei dem der selektierte Teil des Moleküls eine aktivierte Stelle eines Enzyms umfasst.
8. Verfahren zur Herstellung von Antikörpern gemäß einem der vorstehenden Ansprüche, bei dem die Antikörper gegen den nicht-selektierten Teil intravenös injiziert werden.
9. Verfahren zur Herstellung von Antikörpern gemäß Anspruch 4 oder jeden direkt oder indirekt von Anspruch 4 abhängigen Anspruch, bei dem der Antikörper aus dem Blut oder einer anderen Substanz oder einem anderen Material in einer Weise extrahiert wird, die das Tier leben lässt.
10. Verfahren zur Herstellung von Antikörpern, die für Epitope auf leichten kappa- oder lambda-Ketten spezifisch sind, die verborgen sind und die nicht zum Binden im Gesamt-Immunglobulin verfügbar

sind, sondern auf den freien leichten Ketten exponiert sind, umfassend Tolerantmachen des immunologischen Systems einer ersten nicht-menschlichen Quelle von Antikörpern mit Immunglobulin mit gebundener leichter Kette und mit Antikörpern gegen die exponierten Epitope des Immunglobulins, und Immunisieren der ersten Quelle von Antikörpern mit freier leichter Kette, und Gestatten der ersten Quelle von Antikörpern, für die freie leichte Kette spezifische Antikörper zu produzieren, und Extrahieren der von der ersten Quelle produzierten Antikörper; wobei die zweite Quelle, die verwendet wird, um den Antikörper gegen die exponierten Epitope des Immunglobulins zu produzieren, nicht dieselbe individuelle nicht-menschliche Tier-Quelle wie die erste Quelle ist, die später verwendet wird, um den Antikörper gegen die freien Ketten zu produzieren und wobei die zum Produzieren des Antikörpers gegen die freie Kette gewählte erste Quelle nicht zuvor mit dem Immunglobulin immunisiert worden ist.

Revendications

1. Procédé de production d'anticorps dirigés contre une partie sélectionnée d'une molécule comprenant la tolérisation du système immunologique d'une première source animale non humaine d'anticorps à une partie non sélectionnée de ladite molécule par la présentation au système immunologique de la première source, d'un composé contenant la partie non sélectionnée de la molécule, et également par la présentation à la première source, d'anticorps envers la partie non sélectionnée de la molécule, et l'immunisation de la première source d'anticorps avec la molécule entière, c'est-à-dire la partie sélectionnée de la molécule avec les parties de la molécule utilisée pour la tolérisation, en permettant à la première source d'anticorps de produire des anticorps spécifiques à ladite partie sélectionnée de la molécule et en extrayant les anticorps produits à partir de la première source, et dans lequel la deuxième source qui est utilisée pour produire l'anticorps dirigé contre la partie non sélectionnée n'est pas la même première source individuelle qui est utilisée ultérieurement pour produire l'anticorps dirigé contre la partie sélectionnée, et la première source choisie pour produire l'anticorps dirigés contre la partie sélectionnée n'a pas été immunisée envers la partie non sélectionnée auparavant.
2. Procédé de production d'anticorps selon la revendication 1, qui comprend la tolérisation du système immunologique de la première source à la partie non sélectionnée de la molécule par l'immunisation d'une deuxième source animale non humaine d'anticorps avec la partie non sélectionnée ou la molé-

cule entière pour produire un anticorps dirigé contre la partie non sélectionnée, l'extraction de l'anticorps dirigé contre la partie non sélectionnée, et l'utilisation de l'anticorps dirigé contre la partie non sélectionnée pour tolérer une première source d'anticorps afin d'améliorer la production d'anticorps dirigés contre la partie sélectionnée.

3. Procédé selon la revendication 1 ou la revendication 2, dans lequel du sang ou une autre substance produite par la première source animale non humaine est extrait(e) et l'anticorps dirigé contre la partie sélectionnée ou non sélectionnée est extrait dudit sang ou de ladite autre substance. 10
4. Procédé selon l'une quelconque des revendications précédentes, dans lequel les anticorps produits sont des anticorps polyclonaux. 15
5. Procédé selon l'une quelconque des revendications précédentes, dans lequel la partie sélectionnée comprend la différence entre deux molécules similaires, et dans lequel la partie non sélectionnée utilisée pour la tolérification est une molécule entière (ou sensiblement entière) et l'étape d'immunisation est réalisée en introduisant dans la source l'autre molécule entière (ou sensiblement entière). 20 25
6. Procédé de production d'anticorps selon la revendication 1 contre une première sous-classe d'immunoglobulines G du groupe IgG1, IgG2, IgG3 et IgG4, ledit procédé comprenant la tolérification du système immunologique d'une première source non humaine d'anticorps aux autres sous-classes d'immunoglobulines de l'immunoglobuline et également la présentation à la première source, d'anticorps dirigé contre les autres sous-classes d'immunoglobulines de l'immunoglobuline, et l'immunisation de la première source d'anticorps avec la première sous-classe d'immunoglobulines, en permettant à la première source d'anticorps de produire des anticorps spécifiques à ladite sous-classe d'immunoglobulines, et l'extraction des anticorps à partir de la première source. 30 35 40 45
7. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel la partie sélectionnée de la molécule comprend un site activé d'une enzyme.
8. Procédé de production d'anticorps selon l'une quelconque des revendications précédentes, dans lequel les anticorps de la partie non sélectionnée sont injectés par voie intraveineuse. 50
9. Procédé de production d'anticorps selon la revendication 4 ou une quelconque revendication dépendant directement ou indirectement de la revendication 4, dans lequel l'anticorps est extrait du sang ou

d'une autre substance ou matériau de telle façon que l'animal reste en vie.

10. Procédé de production d'anticorps spécifiques d'épitopes des chaînes légères kappa ou lambda qui sont cachés et ne seront pas disponibles pour liaison dans l'immunoglobuline totale mais sont exposés sur des chaînes légères libres tolérant le système immunitaire d'une première source animale non humaine d'anticorps avec de l'immunoglobuline avec une chaîne légère liée et avec des anticorps des épitopes exposés de l'immunoglobuline, et immunisant la première source d'anticorps avec une chaîne légère libre et permettant à la première source d'anticorps de produire des anticorps spécifiques à la chaîne légère libre, et extrayant des anticorps produits à partir de la première source, et dans lequel la seconde source qui est utilisée pour produire l'anticorps des épitopes exposés de l'immunoglobuline n'est pas la même source animale non humaine individuelle que la première source qui est utilisée ensuite pour produire l'anticorps aux chaînes libres, et dans lequel la première source choisie pour produire l'anticorps de la chaîne libre n'a pas été immunisée au préalable avec l'immunoglobuline.

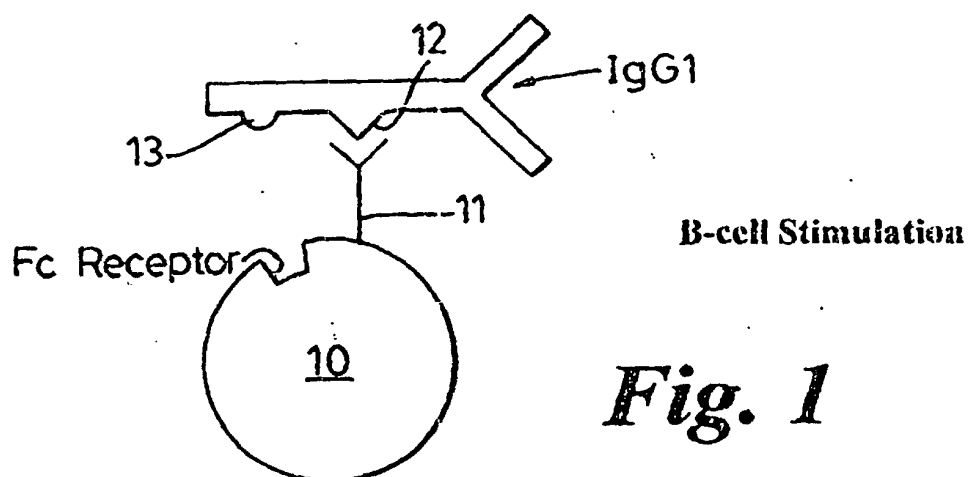


Fig. 1

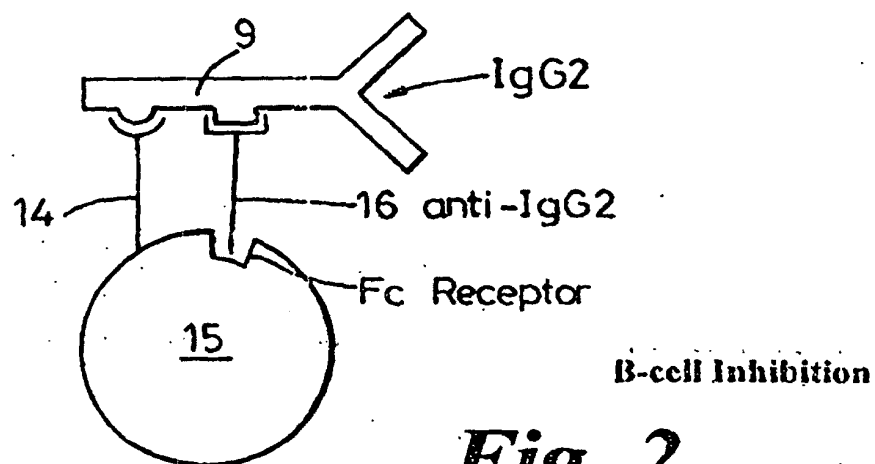


Fig. 2

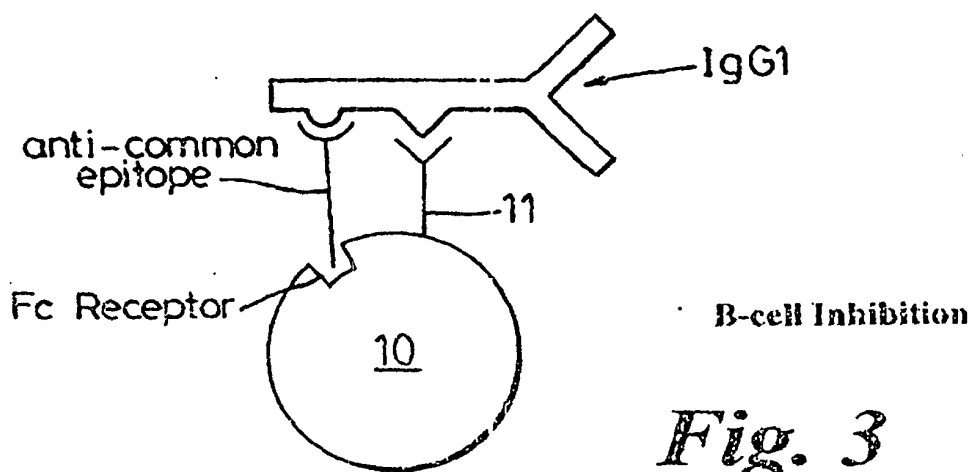


Fig. 3

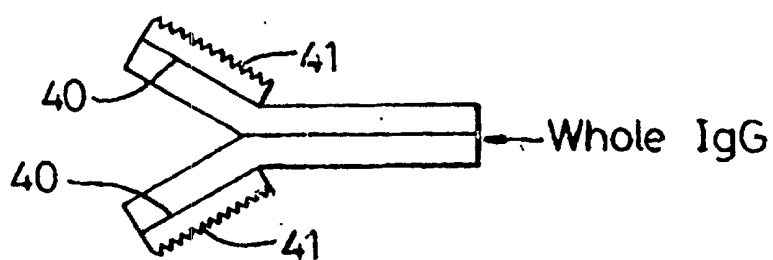
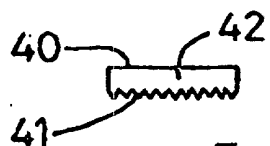
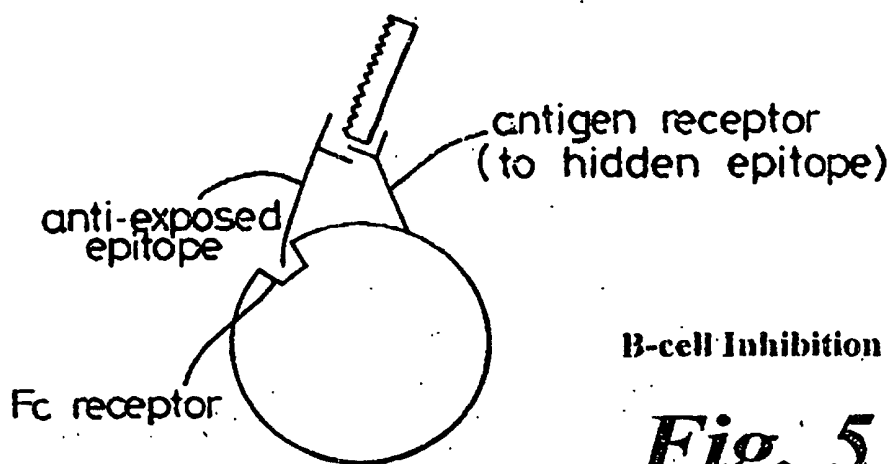


Fig. 4

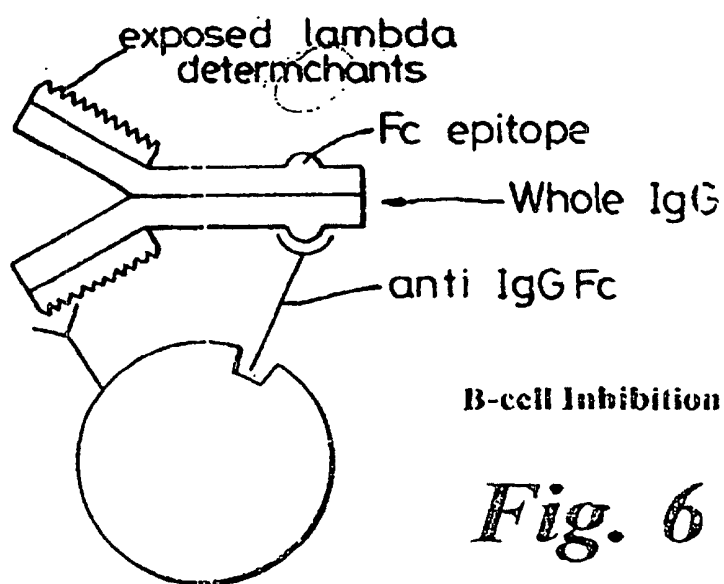


Free lambda



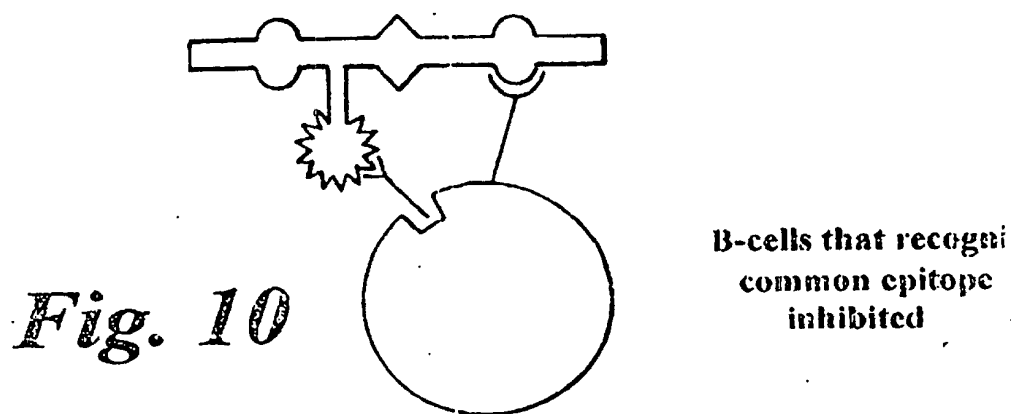
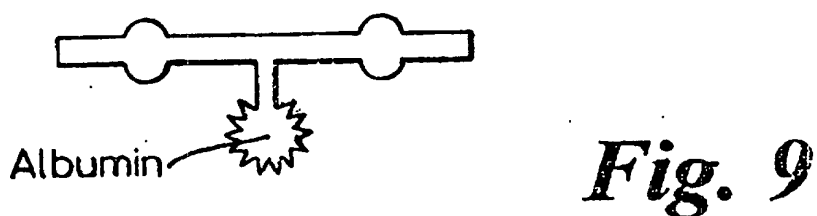
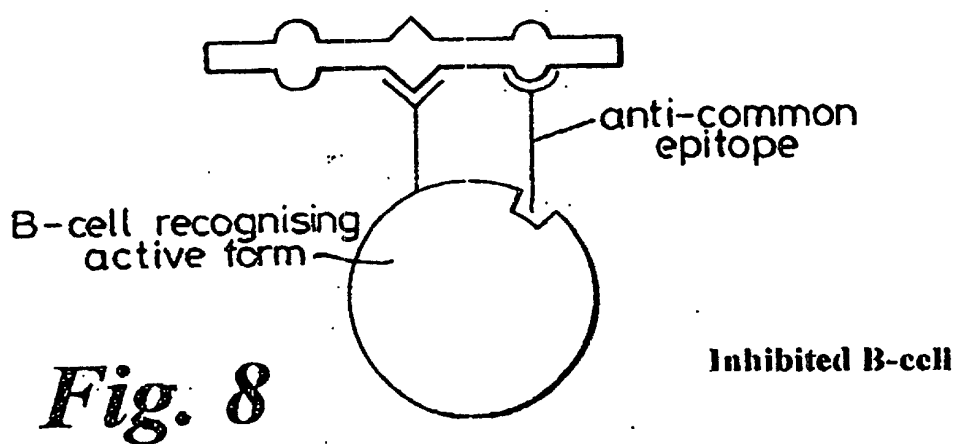
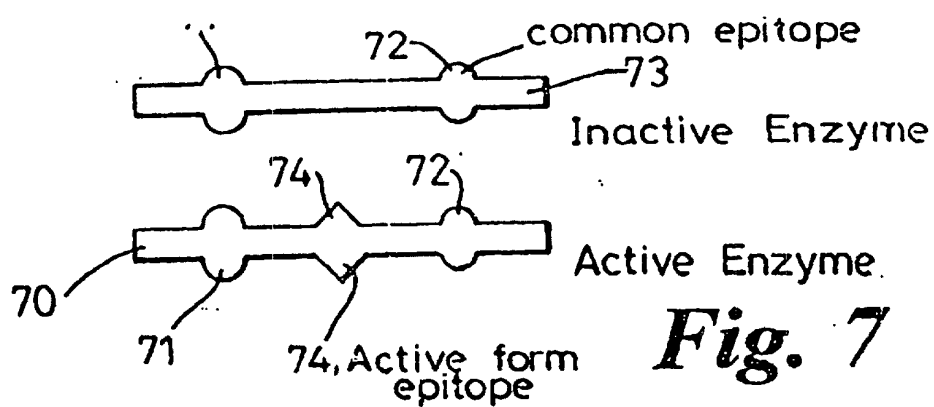
B-cell Inhibition

Fig. 5



B-cell Inhibition

Fig. 6



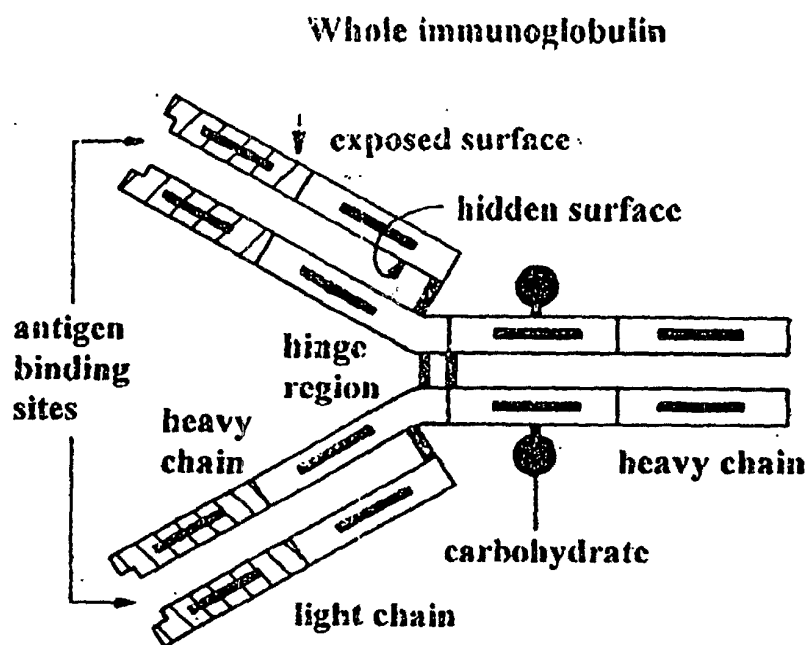


Fig. 11A

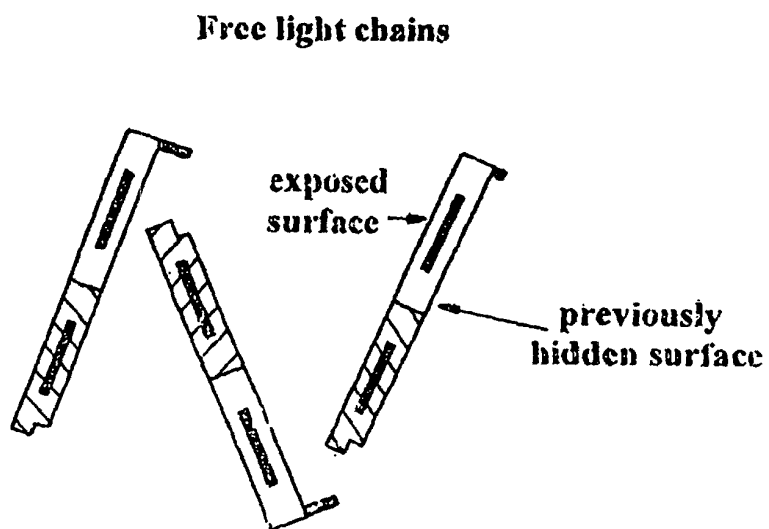


Fig. 11B